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Two species of *Cladosporium* associated with wood discoloration in Korea

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Abstract — During our previous study on the diversity of molds inhabiting wood, many *Cladosporium* isolates were collected and phylogenetically identified. Among them were isolates of two species previously unrecorded from Korea. Here, we confirm the isolates as *Cladosporium perangustum* and *C. ramotenellum* and provide descriptions and illustrations of cultural and microscopic characters on standard media. We draw attention to a broadening of the range of several characters for *C. ramotenellum* and to possible alternative phylogenetic relationships of *C. perangustum* when comparing our multi-gene analysis with previous analyses.

Key words — ascomycetes, phylogeny, taxonomy

Introduction

Cladosporium Link is one of the most widely known (if under-studied) molds. It is a cosmopolitan genus with more than 772 names (Dugan et al. 2004) and recently Bensch et al. (2012) recognized a total of 169 *Cladosporium* species. It has been isolated from air, soil, and many other substrates (Schubert et al. 2007, Bensch et al. 2010, 2012). Members are variously known to be saprobic, endophytic, or plant pathogenic (Schubert et al. 2007, Bensch et al. 2010, 2012). In Korea, about 20 *Cladosporium* species have been reported (Min 1985, Shin & Braun 1995, Shin & Im 1999, Kwon et al. 2000, 2001, Paul & Yu 2008, Korean Society of Plant Pathology 2009, Bensch et al. 2010). Recently Lee et al. (2012) phylogenetically examined *Cladosporium* representatives inhabiting wood products in Korea and recognized nine *Cladosporium* species. Among them were *C. perangustum* and *C. ramotenellum*, not previously recorded in Korea. Here, we confirm their identities by morphology and phylogenetic re-analysis and provide detailed descriptions and figures for these two *Cladosporium* species.

Materials & methods

Morphological examination

Four *Cladosporium* isolates, KUC1462, KUC1767, KUC3027, and KUC5085 studied by Lee et al. (2012) were used in this study. They were inoculated onto SNA (Synthetic nutrient-poor agar: KH_2PO_4 1 g, KNO_3 1 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; KCl 0.5 g; glucose 0.2 g; sucrose 0.2 g; agar 20 g; distilled water 1000 ml) and cultivated at 25°C for 7 days. Preparations were mounted in Shear's solution (CH_3COOK 3 g; distilled water 150 ml; glycerin 60 ml; 95% ethanol 90 ml) according to Crous et al. (2009). Microscopic observations were made using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) and photos were taken using the same microscope and an Axio Imager A1 microscope (Zeiss, Jena, Germany). For determination of cultural characteristics, colonies were cultivated on PDA (Potato dextrose agar, Difco), MEA (Malt extract agar: malt extract 30 g; agar 15 g; distilled water 1000 ml), and OA (Oatmeal Agar, Difco) at 25°C for 14 days in the dark. The isolates were deposited at the National Institute of Biological Resources, Incheon, South Korea (KB) with the acronym KUC, which refers to the Korea University Culture Collections, Korea University, Seoul, South Korea.

Phylogenetic analysis

DNA sequences of four *Cladosporium* isolates (KUC1462, KUC1767, KUC3027, KUC5085) from the internal transcribed spacer (ITS), actin (ACT), and translation elongation factor-1α (TEF) regions were obtained from Lee et al. (2012). ITS, ACT, TEF sequences were aligned separately with the reference sequences from Bensch et al. (2010) using MAFFT 6.885 (Katoh & Toh 2008). The L-INS-I alignment option was used for all datasets. Each dataset was manually edited with MacClade 4.08 (Maddison & Maddison 2005). Gaps were treated as missing. The best-fit model was applied for each dataset using AIC in MrModeltest 2.3 (Nylander 2004) and the three datasets were combined. Bayesian analysis was performed with the combined dataset using MrBayes 3.2.1 (Ronquist et al. 2012). Two independent runs (four chains each) of 705,000 generations were performed and every 100th tree was sampled. The first 25% of sampled trees were discarded and the remaining 75% were used for construction of a 50% major-rule consensus tree. The graphic representation of the likelihood scores of the sampled trees was checked. The standard deviation of split frequencies is below 0.01 and the potential scale reduction factor (PSRF) was close to 1.0. The tree was viewed via FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Taxonomy

Cladosporium perangustum Bensch, Crous & U. Braun, Stud. Mycol. 67: 65. 2010.
Figs 1–2

Mycelium internal and superficial; 1.5–4 µm wide, septate, sometimes slightly constricted at septa, sometimes intercalary swellings and constrictions present, subhyaline or pale olivaceous-brown, smooth or verruculose. Conidiophores macronematous or micronematous arising terminally and laterally from hyphae, straight or slightly curved, filiform to narrowly cylindrical-oblong, unbranched, occasionally branched, conidiophores 14–142 × 2–3.5 µm, pale olivaceous-

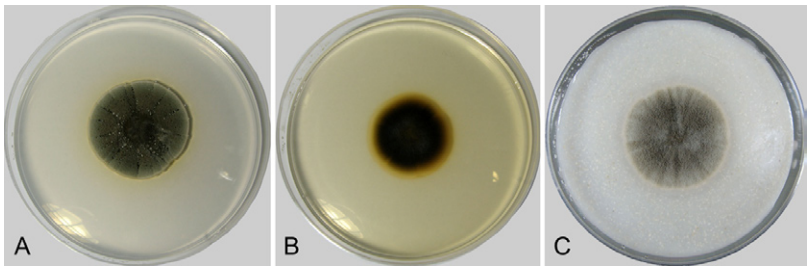

Fig. 1. *Cladosporium perangustum* (KUC1767) grown in 9 cm diam Petri dishes for 14 d at 25°C. A: PDA. B: MEA. C: OA.

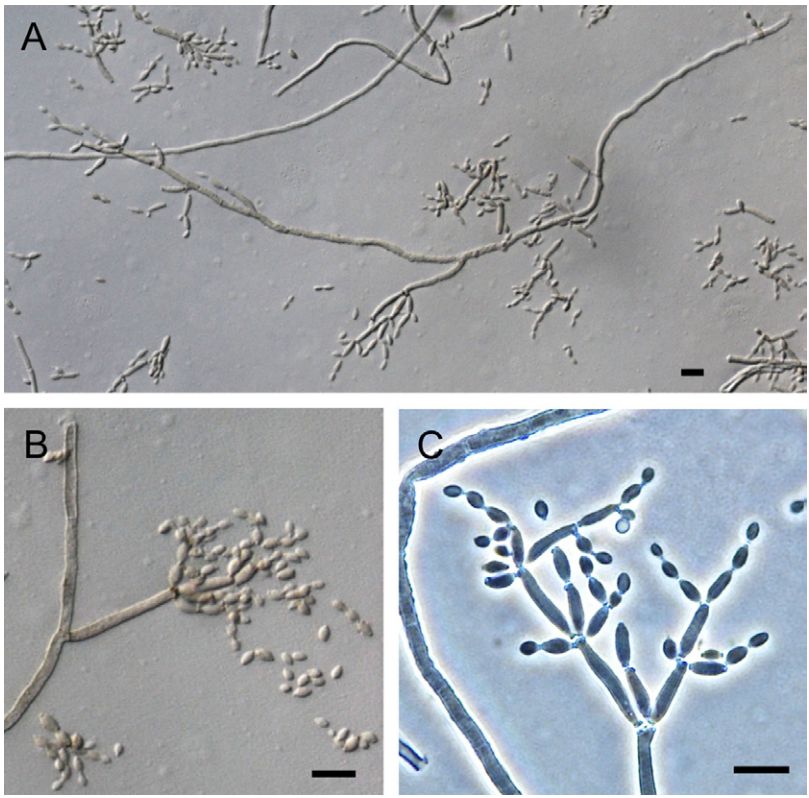

Fig. 2. *Cladosporium perangustum*. A, B. Macro- and micronematous conidiophores (KUC1462). C. Ramoconidia and conidia (KUC1767). Scale bar = 10 µm.

brown, asperulate-verruculose towards the base of conidiophores, at the apex smooth, sometimes slightly attenuated towards the apex. Conidiogenous cells integrated, mainly terminal, sometimes also intercalary, narrowly cylindrical-

oblong, in intercalary cells loci situated on small peg-like lateral prolongations or just below the septum, 13–36 μm long, with 1–4 apically crowded loci. Ramoconidia cylindrical-oblong, up to 44 μm long, 2.5–3.5 μm wide, rarely septate, base truncate, secondary ramoconidia narrowly ellipsoid to cylindrical-oblong, 8–21 × 2–3 μm (av. ± SD: 13.7 ± 4.1 × 2.5 ± 0.2), with 2–4 distal hila, pale olivaceous-brown. Conidia numerous, catenate, in branched chains, branching in all directions, 1–4 conidia in the terminal unbranched part of the chain, small terminal conidia globose, subglobose or ovoid to obovoid, 2–3.5 × 1.5–2 μm (av. ± SD: 2.9 ± 0.4 × 1.8 ± 0.2), intercalary conidia ovoid, limoniform to ellipsoid, somewhat fusiform, 4–8.5 × 2–3 μm (av. ± SD: 5.9 ± 1.4 × 2.2 ± 0.2), attenuated towards apex and base, with 1-3 distal hila, pale olivaceous-brown.

Culture characteristics Colonies on PDA attaining 32–38 mm diam after 14 d at 25°C, greenish grey to dull green, reverse olive or dark green, fluffy, floccose or powdery, margins glabrous, whitish, olive grey or pale regular or somewhat undulate, aerial mycelium loosely floccose or felty, occasionally numerous small to large prominent exudates formed, sporulation abundant. Colonies on MEA reaching 20–29 mm diam after 14 d at 25°C, dark green or brown, reverse olive brown, velvety to floccose, margins white to light yellow, narrow to broad, regular to undulate, glabrous, aerial mycelium abundantly formed, sometimes covering most parts of colony surface, loosely to densely floccose or felty, growth habit plane to sometimes elevated, sporulation abundant. Colonies on OA 35–49 mm diam after 14 d at 25°C, grey to dark green, fluffy to felty-floccose, margins colorless or white, up to 2 mm diam, aerial mycelium abundant, covering large parts of the colony surface, dense, low to high, white, sporulating profusely.

Specimens examined: **KOREA**, **Gyeongsangbuk-do**, Bonghwa-gun, on the surface of *Pinus densiflora* log, 05 June 2001 (KB, KUC1462; GenBank JN033481, JN033508, JN033536); **Gyeongsangnam-do**, Pusan, on the surface of *Pinus radiata* log, August 2000 (KB, KUC1767; GenBank JN033468, JN033495, JN033523); **Incheon**, on the log surface of *Picea abies*, 16 June 2007 (KUC5085; GenBank JN033460, JN033487, JN033515).

Remarks — The colony morphology and microscopic features of the isolates agreed well with the description of *C. perangustum* (Bensch et al. 2010). The fungus has been reported from Africa, Asia, Australasia, Europe, and North America. According to Bensch et al. (2010), *Cladosporium exile* Bensch et al. and *C. scabrellum* Bensch et al. are similar to *C. perangustum* but *C. exile* has longer and wider conidiophores, wider ramoconidia and conidia, and shorter intercalary conidia. *Cladosporium scabrellum* differs in having mainly macronematous, wider conidiophores, and wider secondary ramoconidia. Compared to the other species studied by Lee et al. (2012), *C. perangustum* showed medium discoloration on *Pinus densiflora* and *Pinus radiata*.

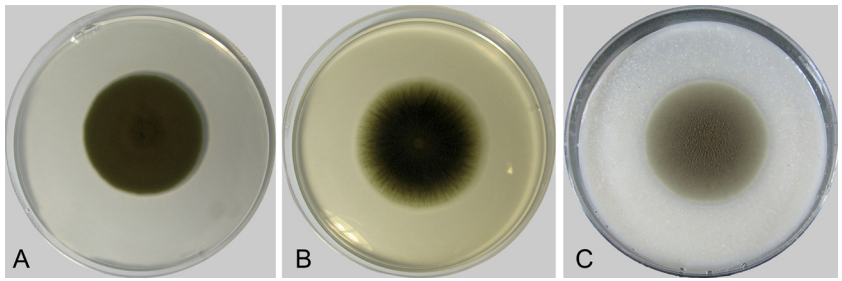

Fig. 3. *Cladosporium ramotenellum* (KUC3027) grown in 9 cm diam Petri dishes for 14 d at 25°C. A: PDA. B: MEA. C: OA.

Cladosporium ramotenellum K. Schub., Zalar, Crous & U. Braun, Stud. Mycol. 58: 137. 2007. Figs 3–4

Mycelium internal and superficial, 1.5–4 µm wide, septate, without swellings and constrictions, hyaline or subhyaline, smooth. Conidiophores macronematous and micronematous, arising terminally or laterally from branches of plagiotropous hyphae, straight or slightly flexuous, oblong or cylindrical, unbranched, sometimes branches often only as short lateral prolongations, 15–117 × 2–3.5 µm, septate, pale olivaceous-brown or brown, smooth to verruculose. Conidiogenous cells integrated, terminal, sometimes also intercalary, cylindrical, 15–49 µm long, proliferation sympodial, with few conidiogenous loci, mostly 1–3, loci sometimes situated on small lateral prolongations. Ramoconidia cylindrical-oblong, up to 46.5 µm long, 2–3.5 µm wide, usually 0–1-septate, pale olivaceous, smooth or verruculose, with a broadly truncate base, secondary ramoconidia subcylindrical to cylindrical-oblong, 12.5–22 × 2–4 µm (av. ± SD: 18 ± 4 × 3.1 ± 0.5), pale olivaceous, apex broadly rounded or slightly attenuated towards apex and base. Conidia numerous, catenate, in branched chains, branching in all directions, small terminal conidia numerous, 1–3 conidia in the terminal unbranched part of

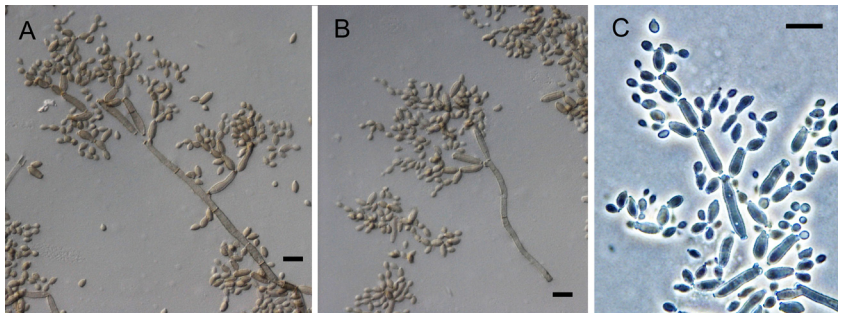

Fig. 4. *Cladosporium ramotenellum* (KUC3027). A, B. Macro- and micronematous conidiophore. C. Ramoconidia and conidia. Scale bars = 10 µm.

the chain, globose, subglobose or ovoid, obovoid, 3.5–7 × 2–3 μm (av. ± SD: 4.3 ± 0.7 × 2.5 ± 0.3), intercalary conidia ellipsoid to subcylindrical, or limoniform, 4–11 × 2.5–4(–4.5) μm (av. ± SD: 6.6 ± 1.6 × 3 ± 0.3).

Culture characteristics Colonies on PDA reaching 41–47 mm diam after 14 d at 25°C, olive due to abundant sporulation, margins entire, white, glabrous, aerial mycelium absent or sparse, growth flat with a somewhat folded and wrinkled colony center, no exudates, sporulation profuse. Colonies on MEA reaching 46–49 mm diam after 14 d at 25°C, olive, velvety, greyish green to dark green in reverse, margins entire, colorless, glabrous to feathery, aerial mycelium sparse, diffuse, growth flat with slightly elevated colony center, prominent exudates not formed, abundantly sporulating. Colonies on OA attaining 39–41 mm diam after 14 d at 25°C, olivaceous, margin entire, colorless or white, aerial mycelium sparse, growth flat, without exudates, sporulation profuse.

Specimen examined: **KOREA**, on the surface of Chromated Copper Arsenate (CCA)-treated wood product (*Pinus radiata*), 2003 (KUC3027; GenBank JN033464, JN033491, JN033519).

Remarks — The colony morphology and microscopic features of our isolate agreed well with the description of *C. ramotenellum* (Schubert et al. 2007), although it has smaller intercalary conidia and secondary ramoconidia. The fungus previously has been reported only from Slovenia. According to Schubert et al. (2007), *C. ramotenellum* resembles *C. cladosporioides* (Fresen.) G.A. de Vries and *C. tenellum* K. Schub. et al. but differs from *C. cladosporioides* by having narrower conidiophores and conidia. *Cladosporium tenellum* possesses conidiophores with numerous conidiogenous loci, and shorter and wider conidia. Also, *C. ramotenellum* grows faster in culture than *C. tenellum*. *Cladosporium ramotenellum* showed a discoloration rate similar to *C. perangustum* on *Pinus densiflora*, but a little bit more prominent than *C. perangustum* on *Pinus radiata* (Lee et al. 2012).

Phylogeny

The resulting alignment contained 36 taxa (including the outgroup taxon) and 481, 139 and 201 characters (including alignment gaps) were used in the ITS, ACT and TEF partitions, respectively. The model used for ITS was SYM+G model. For ACT and TEF, HKY+I+G model and GTR+G model were used, respectively. For the Bayesian analysis, 10,705 trees were obtained from which the consensus tree and posterior probabilities were calculated (Fig. 5). The species relationships in the tree were sometimes not as well resolved as in previous studies, or resolved differently (Bensch et al. 2010, Bensch et al. 2012). The phylogenetic analysis revealed that *Cladosporium perangustum* isolates, KUC1462, KUC1767 and KUC5085 were clustered within the *C. perangustum* species clade with a high posterior probability value (1.0

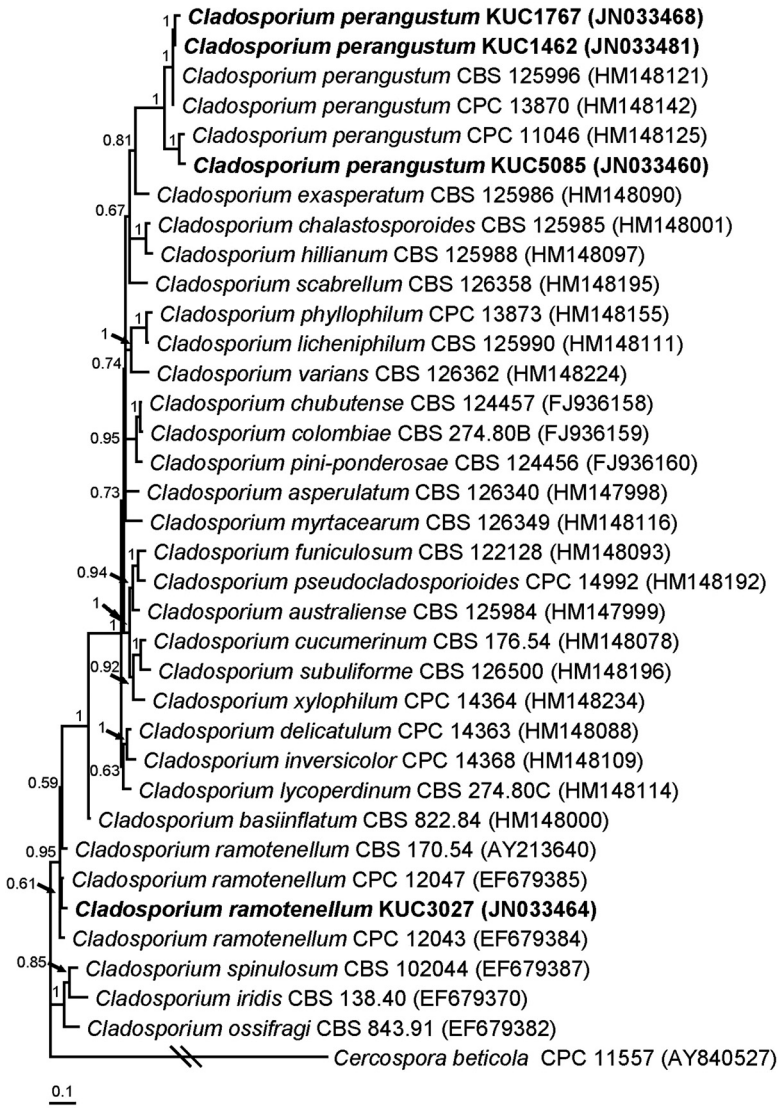
Cladosporium perangustum KUC1767 (JN033468)
Cladosporium perangustum KUC1462 (JN033481)
Cladosporium perangustum CBS 125996 (HM148121)
Cladosporium perangustum CPC 13870 (HM148142)
Cladosporium perangustum CPC 11046 (HM148125)
Cladosporium perangustum KUC5085 (JN033460)
Cladosporium exasperatum CBS 125986 (HM148090)
Cladosporium chalastosporoides CBS 125985 (HM148001)
Cladosporium hillianum CBS 125988 (HM148097)
Cladosporium scabrellum CBS 126358 (HM148195)
Cladosporium phyllophilum CPC 13873 (HM148155)
Cladosporium licheniphilum CBS 125990 (HM148111)
Cladosporium varians CBS 126362 (HM148224)
Cladosporium chubutense CBS 124457 (FJ936158)
Cladosporium colombiae CBS 274.80B (FJ936159)
Cladosporium pini-ponderosae CBS 124456 (FJ936160)
Cladosporium asperulatum CBS 126340 (HM147998)
Cladosporium myrtacearum CBS 126349 (HM148116)
Cladosporium funiculosum CBS 122128 (HM148093)
Cladosporium pseudocladosporioides CPC 14992 (HM148192)
Cladosporium australiense CBS 125984 (HM147999)
Cladosporium cucumerinum CBS 176.54 (HM148078)
Cladosporium subuliforme CBS 126500 (HM148196)
Cladosporium xylophilum CPC 14364 (HM148234)
Cladosporium delicatulum CPC 14363 (HM148088)
Cladosporium inversicolor CPC 14368 (HM148109)
Cladosporium lycoperdinum CBS 274.80C (HM148114)
Cladosporium basiinflatum CBS 822.84 (HM148000)
Cladosporium ramotenellum CBS 170.54 (AY213640)
Cladosporium ramotenellum CPC 12047 (EF679385)
Cladosporium ramotenellum KUC3027 (JN033464)
Cladosporium ramotenellum CPC 12043 (EF679384)
Cladosporium spinulosum CBS 102044 (EF679387)
Cladosporium iridis CBS 138.40 (EF679370)
Cladosporium ossifragi CBS 843.91 (EF679382)
Cercospora beticola CPC 11557 (AY840527)
0.1

Fig. 5. 50% majority-rule consensus tree of 10,575 trees resulting from a bayesian analysis of 36 sequences in a combined ITS, ACT, and TEF alignment. Bayesian posterior probabilities ≥50% are shown. The tree was rooted to sequences of *Cercospora beticola* strain CPC 11557. GenBank Accession numbers of ITS sequences are shown in parentheses.

p.p.). In our analysis, *C. perangustum* was sister to *C. exasperatum* Bensch et al., a result differing substantially from the analysis of Bensch et al. (2010). In our analysis, *Cladosporium ramotenellum* KUC3027 was monophyletic with *C. ramotenellum* CPC 12047, but not with *C. ramotenellum* CPC 12043 and CBS 170.54. Also the monophyly of *C. ramotenellum* KUC3027 and CPC 12047 was weakly supported (61% posterior probability value). Nevertheless, the cultural and microscopic observations of our *C. ramotenellum* KUC3027 were consistent with the descriptions of *C. ramotenellum* (Schubert et al. 2007; Bensch et al. 2010).

Acknowledgments

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